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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 120122.405PC	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2005/008870	International filing date *(day/month/year)* 16 March 2005 (16.03.2005)	Priority date *(day/month/year)* 16 March 2004 (16.03.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant AMNIS CORPORATION		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 19 September 2006 (19.09.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Ellen Moyse e-mail: pt05@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

PCT 2915

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/US2005/008870	International filing date *(day/month/year)* 16.03.2005
Priority date *(day/month/year)* 16.03.2004	
International Patent Classification (IPC) or both national classification and IPC G01N15/14, G01N21/64, G01N33/50	
Applicant AMNIS CORPORATION	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Duijs, E Telephone No. +49 89 2399-7945

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/008870

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: US 2004/021868 A1 (ORTYN WILLIAM E ET AL)
   D2: WO 95/20148 A (COULTER CORPORATION)

2. **Novelty (Art. 33(2) PCT) and Inventive Step (Art. 33(3) PCT):**

   The present application does not meet the criteria of Article 33(1) PCT,:

   The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claims 1-23 and 25-27** is not new in the sense of Article 33(2) PCT, and because the subject-matter of **claims 1-28** does not involve an inventive step in the sense of Article 33(3) PCT:

2.1 **Independent method claim 1:**

D1 discloses (the references in parentheses applying to this document):
-- A method for identifying a specific cell (par. 138: "cell identification");
-- directing incident light 58 at a cell (fig. 5; par. 78-80);
-- using a (TDI) detector 44 to obtain a **side scatter** image (fig. 5; par. 78-80: "collection lens 32 is at about 90° angle relative to the directions of the light incident"; par. 135: "dark field");
-- and using the spatial frequency content (par. 63) of the **side scatter** image to identify a specific cell (par. 135: "cell viability and apoptosis staging, and necrosis differentiation"; par. 136: "cell analysis and classification"; par. 138: "cell identification").
Hence, claim 1 is not new (Art. 33(2) PCT).

2.2 **Independent method claim 8:**

D1 discloses (the references in parentheses applying to this document):

-- A method for identifying a specific cell (par. 138: "cell identification");
-- directing incident light 58 at a cell (fig. 5; par. 78-80);
-- using a (TDI) detector 44 to obtain a **brightfield** image (fig. 5; par. 79-80: "light source 62... generally aligned with the optical axis of the collection lens", "light source 64... reflected"; par. 135: "brightfield");
-- and using the spatial frequency content (par. 63) of the **brightfield** image to identify a specific cell (par. 135: "cell viability and apoptosis staging, and necrosis differentiation"; par. 136: "cell analysis and classification"; par. 138: "cell identification").
Hence, claim 8 is also not new (Art. 33(2) PCT).

2.3 **Independent method claim 16:**
D1 discloses (the references in parentheses applying to this document):
-- A method for identifying a specific cell (par. 138: "cell identification");
-- contacting a cell with a **nuclear marker** (par. 121: "cell nuclei 202 stained with a green fluorescent dye");
-- directing incident light 58 at the marked cell (fig. 5; par. 80: "light source 66 to excite fluorescence");
-- using a (TDI) detector 44 to obtain an image of the cell (par. 80; par. 121: "images of cell nuclei 202 stained with a green fluorescent dye"; par. 135: "fluorescent images");
-- and using the **nuclear marker** image in combination with the spatial frequency content (par. 63) of the cell image to identify a specific cell (par. 135: "cell viability and apoptosis staging, and necrosis differentiation"; par. 136: "cell analysis and classification"; par. 138: "cell identification").
Hence, claim 16 is also not new (Art. 33(2) PCT).

2.4 **Independent apparatus claim 27:**
What has been said above with reference to claim 16 concerns claim 27 mutatis mutandis.

2.5 **Dependent claims 2-7, 9-15, 17-26 and 28** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and or inventive step, see **D1** and the corresponding

passages cited in the search report. See also:

- **Claims 2, 9, 17**: relative motion (D1: par. 66, "fluid flow 22", par. 69);
- **Claims 3, 10, 18**: heterogeneous cell population (par. 135: analyzing tens of thousands of cells, rare cell detection... differentiation; par. 107, male/female cells);
- **Claims 4, 11, 19**: apoptotic cell (D1: par. 135);
- **Claims 5, 12, 20**: early or late stage apoptotic cell (D1: par. 135);
- **Claims 6, 13, 21**: necrotic cell (D1: par. 135);
- **Claims 7, 14, 22**: at least one of an apoptotic cell and a necrotic cell (D1: par. 135);
- **Claim 15**: spatial frequency content of the nucleus (D1: par. 63, 121; par. 107: "TDI detector 44 also distinguishes the spatial potion");
- **Claim 23**: single nuclear marker (D1: par. 121, "cell nuclei 202 stained with a green fluorescent dye");
- **Claim 25**: images are collected simultaneously (D1: par. 135, "six channels of multimode imagery");
- **Claim 26**: TDI detector (D1: par. 66, "44").

Hence, claims 2-7, 9-15, 17-23, 25 and 26 are also not new (Art. 33(2) PCT).

- D1 discloses the use of a nuclear marker (par. 121: "cell nuclei 202 stained with a green fluorescent dye"). The use of 7-aminoactinomycin D (7-ADD), as defined in **claims 24 and 28** cannot be regarded as involving an inventive step, since the marker 7-ADD is merely one of several known (see **D2**, p. 7, l. 35-37), commercial available possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill.

3. Industrial applicability (Article 33(4) PCT):

The requirement of Art. 33(4) PCT as to industrial applicability is fulfilled for all claims.

Re Item VII
Certain defects in the international application (form or content)

4.1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, **the relevant background art** disclosed in the **document D1** is not mentioned in the description, nor is this document identified therein.

4.2 The used expression "**incorporated herein by reference**, in their entirety" on page 11, l. 3-6, or any expression of the same kind has to be deleted from the description. Only if matter in the document referred to is essential to satisfy the requirements of Art. 5 PCT, it has to be expressly incorporated into the description, because the patent specification should, regarding the essential features of the invention, be self-contained (see the PCT Guidelines, II, 4.17).

Re Item VIII
Certain observations on the international application (clarity)

5.1 Although **apparatus/system claim 27** and **method claims 1, 8 and 16** have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore **lack conciseness**.
Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection.
Hence said claims do not meet the requirements of Article 6 PCT.

It appears to be possible to define the relevant subject-matter in terms of a minimum number of independent claims in each category followed by dependent claims covering features which are merely optional (Rule 6.4 PCT).

5.2 The term "**spatial frequency content**" used in **claims 1, 8, 15 and 16** is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims

unclear (Article 6 PCT).
According to the description (p. 7, l. 19-21), the "spatial frequency content" is one out of a plurality of "morphological parameters". It appears that every image can be Fourier transformed to extract a "spatial frequency content". At present, the scope of the claims should be regarded as being merely limited to "using the side scatter / brightfield / nuclear marker image to identify a specific cell".

5.3 It is unclear (Art. 6 PCT) in **independent claims 1, 8 and 16**, **how** a cell can be identified from the side scatter **or** brightfield **or** nuclear marker images. It appears from the description (example 11), that in order to be able to distinguish (identify) all four cell populations, morphologic features derived from side scatter (darkfield) **and** brightfield **and** nuclear marker (7-ADD) images have to be used.

5.4 It is unclear (Art. 6 PCT) in **claim 16**, what the difference between the "nuclear marker image" and the "cell image" is.

5.5 The vague and imprecise statement ("**spirit**... of the invention") in the description on page 19, l. 9-12 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

5.6 The **dependency of claim 24** is unclear (Art. 6 PCT), since claim 24 is dependent on claim 16 and refers to "the single nuclear marker", which is introduced for the first time in claim 23. Hence claim 24 should be dependent on claim 23.